Bollworm (Lepidoptera: Noctuidae) Survival on 'Bollgard' and 'Bollgard II' Cotton Flower Bud and Flower Components

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ABSTRACT Genetically modified cotton, *Gossypium hirsutum* L., cultivars ('Bollgard') that produce crystalline proteins from *Bacillus thuringiensis* (Berliner) are valuable tools for managing lepidopteran insect pests in the United States. However, high numbers of bollworm, *Helicoverpa zea* (Boddie), larvae have been observed feeding in white flowers of these cultivars. Fresh tissue bioassays were conducted to investigate bollworm survival on Bollgard and 'Bollgard II' cottons. Bollworm survival was higher on square and flower anthers than on other floral structures on 'Deltapine 5415' (conventional cotton) and 'NuCOTN 33B' (Bollgard). Bollworm survival at 72 h was higher on all floral structures from Deltapine 5415 than on corresponding structures from NuCOTN 33B. ELISA tests indicated that CryIA(c) expression varied among plant parts; however, bollworm survival did not correlate with protein expression levels. Trends in bollworm survival on Bollgard II were similar to those on Bollgard and conventional cotton; however, survival was lower on all structures of Bollgard II than on corresponding structures of Bollgard and conventional cotton. These data support field observations of bollworm injury to white flowers and small bolls and provide a better understanding of larval behavior on Bollgard cotton.

KEY WORDS *Helicoverpa zea*, cotton, genetically modified, Bollgard, Bollgard II

INSECT PEST MANAGEMENT in cotton has traditionally relied upon synthetic insecticides to maintain insect populations below established economic injury levels (Graves et al. 1999). However, insect resistance to insecticides and increasing insecticide costs have made effective and economical insect control difficult. During the last two decades, before widespread resistance, organophosphate and pyrethroid insecticides provided good control of most insect pests of cotton. Currently, these compounds do not provide the same level of protection as they previously did (Graves et al. 1999).

The bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), are primary insect pests of cotton throughout much of the United States. Bollworms and tobacco budworms were highly susceptible to pyrethroid insecticides through the mid-1980s. However, widespread indiscriminate use of these insecticides has resulted in a decline in pyrethroid efficacy against tobacco budworms throughout the United States (Graves et al. 1999) and against bollworms in South Carolina (Brown et al. 1997, Walker et al. 1998). In Louisiana, pyrethroids were recently removed from the list of insecticides recommended by the Louisiana Cooperative Extension Service for tobacco budworm control (Bagwell et al. 2000). Consequently, novel approaches for controlling these insects are being developed (Greenplate et al. 2000a).

Genetically modified cotton cultivars ('Bollgard') that produce *Bacillus thuringiensis* Berliner insecticidal proteins have replaced or supplemented the insecticide component of integrated pest management (IPM) programs throughout the cotton production regions of the United States. Since the introduction of Bollgard cotton in 1996, acreages planted to these cultivars have increased annually. In Louisiana, the percentage of acres planted to Bollgard cotton has increased from ≈15% in 1996 (Williams 1997) to over 60% in 1999 (Williams 2000). Similar trends have been observed in other states, whereas the acreage has decreased in few states (Williams 2000).

Bollgard cotton consistently provides satisfactory control of tobacco budworms. However, bollworms are inherently more tolerant to the protein produced by these cultivars than are tobacco budworms (MacIntosh et al. 1990, Luttrell et al. 1999). Consequently, insecticides are often applied to Bollgard cotton to suppress bollworm populations during peak ovipositional periods (Bacheler and Mott 1997; Layton et al. 1997, 1998; Leonard et al. 1997, 1998; Roof and Durant 1997; Smith 1997, 1998). Burd et al. (1999) found that yields of several commercial Bollgard cotton cultivars were significantly increased when pyrethroids were applied. Because bollworms are readily controlled with pyrethroids, the improvement in yields observed by Burd et al. (1999) may have been the result of bollworm control with the pyrethroids.

Bollworms are more often found in white flowers than other plant parts (Smith 1998, Pietrantonio and

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Heinz 1999). During the first year of commercial Bollgard production, large numbers of bollworm larvae were observed feeding on white flowers in many Bollgard fields across the United States. White flowers of Bollgard cotton appear to be the plant structures most susceptible to bollworm feeding. Gore et al. (2000) infested white flowers and various aged bolls with first instar bollworm larvae. Abscission rates of Bollgard bolls that were infested as white flowers were higher compared with bolls that were infested during later stages of development.

Unacceptable control of bollworms and other lepidopteran pests such as beet armyworms [*Spodoptera exigua* (Hübner)], fall armyworms [*S. frugiperda* (J. E. Smith)], and soybean loopers [*Pseudoplusia includens* (Walker)] prompted scientists with Monsanto Co. to develop a new genetically modified cotton ('Bollgard II') that contains two separate crystalline proteins (Greenplate et al. 2000b). Bollgard II cotton was devoloped by incorporating the CryIIA(b) protein from *B. thuringiensis* into a commercially available Bollgard cotton cultivar, 'Deltapine 50B', which contains the CryIA(c) protein (Greenplate et al. 2000a, 2000b). The CryIIA(b) protein was added to provide greater insecticidal activity against target pests and broaden the spectrum of total pests controlled. A three- to six-fold increase was observed in bioactivity of Bollgard II compared with Bollgard against tobacco budworm (Greenplate et al. 2000b).

The addition of CryIIA(b) protein expressed in Bollgard II cotton provides satisfactory control of beet armyworms, fall armyworms, and soybean loopers (Stewart and Knighten 2000). Also, efficacy of Bollgard II was improved over Bollgard against bollworms (Stewart and Knighten 2000). Other investigators observed improved bollworm control in Bollgard II cotton compared with Bollgard cotton during 1999 (Jackson et al. 2000, Ridge et al. 2000). These initial data indicate that Bollgard II will be beneficial in areas where multiple lepidopteran pest species reach economically damaging levels during most years. However, more research is needed to determine if satisfactory bollworm control will consistently occur in Bollgard II cotton.

Currently, little information is available on why bollworms are more commonly observed on white flowers compared with other plant parts. Possible explanations for differences in bollworm survival may include lower expression of the protein and/or lower levels of secondary plant chemicals in white flowers. Also, the nutritional value of white flowers may be such that bollworm larvae are capable of overcoming the adverse effects of CryIA(c) toxicity. The study reported here used two separate experiments to investigate these possibilities. The first experiment was initiated to determine the levels of bollworm survival that can be expected on white flowers of Bollgard cotton and to determine protein expression levels of white flowers. In the second experiment, white flowers from Bollgard II cotton were evaluated to determine if bollworm control would be significantly improved over that for Bollgard cotton.

Materials and Methods

Bollworm Survival on Floral Components of Conventional and Bollgard Cotton. Plots of a genetically modified cotton cultivar ('NuCOTN 33B', Delta and Pine Land, Scott, MS) producing an insecticidal protein from *Bacillus thuringiensis* Berliner variety *kurstaki* (Bollgard, Monsanto, St. Louis, MO.) and a parental cultivar (Deltapine 5415) were planted from two through 21 May at the Macon Ridge location of the Northeast Research Station near Winnsboro, LA, during 1998 and 1999. Fertilization rates and general agronomic practices followed current Louisiana Cooperative Extension Service recommendations.

Bollworm colonies were established with larvae collected from clover, *Trifolium* spp., during late April and from sweet corn, *Zea mays* L., during early June of each year. Bollworms were reared in the laboratory for a minimum of one generation to eliminate parasitoids, minimize pathogens, and to obtain sufficient numbers of larvae for bioassays. Larvae were fed an artificial soy protein, wheat germ-based diet (*Heliothis* premix, Stonefly Industries, Bryan, TX) in individual 29.5-ml plastic cups (Solo Co., Urbana, IL) with matching lids. Larvae were maintained at 27 ± 2°C, 85 ± 2% RH, and a photoperiod of 14:10 (L:D) h until pupation. Pupae were placed into 3.79-liter cylindrical cardboard containers at 27 ± 2°C and 85 ± 2% RH. Upon eclosion, moths were fed a 10% sucrose:water solution. A single layer of cheesecloth was placed on top of each container to provide an adequate surface for oviposition. Oviposition sheets were harvested daily and placed into 118 by 59 by 354-cm plastic bags until larval eclosion.

Flower buds (squares) and white flowers were harvested from conventional and Bollgard cottons and transported to the laboratory during three stages of cotton plant reproductive development. Cotton plant stages were determined by counting the number of main stem nodes between the upper-most first position white flower and the last unfolded leaf in the plant terminal. Plant stages included main stem nodes above white flower 8–9, 6–7, and 4–5. Floral components included whole squares with the bracts removed, immature reproductive organs (square anthers), white flower bracts, white flower petals, and mature reproductive organs (flower anthers). Flower anthers and square anthers also included the female style and stigma. These structures were placed into 9.0-cm petri dishes along with moistened filter paper. Five neonate bollworm larvae were transferred to each dish and allowed to feed for 72 h. Five dishes were infested per treatment per block ($n = 100$ larvae per treatment). Treatments were arranged in a randomized complete block design (blocks were infested on four successive days). Larval mortality was rated at 24, 48, and 72 h after initial exposure. Percentage survival data within each cultivar was subjected to repeated measures analysis of variance (ANOVA) (SAS Institute 1989), and means were separated according to Fisher's protected least significant difference (LSD). Individual comparisons were made between structures of Nu-

Table 1. Comparisons of bollworm survival at 24, 48, and 72 h after infestation with neonates on Deltapine 5415 and NuCOTN 33B floral components

Hours after infestation	Floral structure	Mean ± SD % survival		df	*t*	*P* > *t*
		DP 5415	NuCOTN 33B			
24						
	Bracts	93 ± 8A	85 ± 15B	30	−1.82	0.08
	Petals	96 ± 4ABC	94 ± 7A	30	−1.23	0.23
	Flower anthers	98 ± 5AB	97 ± 7A	30	−0.40	0.69
	Square anthers	100 ± 0A	97 ± 10A	30	−1.34	0.19
	Squares	95 ± 6BC	93 ± 8A	30	−1.01	0.32
	F	4.37	3.94			
	df	4, 75	4, 75			
	P > *F*	<0.01	0.01			
48						
	Bracts	81 ± 16C	57 ± 21D	30	−3.74	<0.01
	Petals	89 ± 12BC	82 ± 13B	30	−1.53	0.14
	Flower anthers	98 ± 5A	96 ± 4A	30	−0.90	0.38
	Square anthers	98 ± 6A	94 ± 10A	30	−1.32	0.20
	Squares	91 ± 8AB	70 ± 21C	30	−3.71	<0.01
	F	7.20	18.9			
	df	4, 75	4, 75			
	P > *F*	<0.01	<0.01			
72						
	Bracts	71 ± 18C	19 ± 15D	30	−8.76	<0.01
	Petals	76 ± 12BC	58 ± 15B	30	−3.67	<0.01
	Flower Anthers	97 ± 5A	91 ± 6A	30	−2.59	0.01
	Square Anthers	96 ± 6A	88 ± 9A	30	−2.87	0.01
	Squares	83 ± 12B	37 ± 23C	30	−7.12	<0.01
	F	15.8	71.3			
	df	4, 75	4, 75			
	P > *F*	<0.01	<0.01			

Means within columns followed by a common letter are not significantly ($\alpha = 0.05$) different according to Fisher's protected least significant difference. Means within rows are compared using paired *t*-tests ($\alpha = 0.05$).

COTN 33B and Deltapine 5415 using paired *t*-tests from bioassays conducted in 1998 and 1999 (SAS Institute 1989).

Enzyme linked immunosorbent assays (ELISA) were conducted at the United States Department of Agriculture –Agricultural Research Service, Southern Insect Management Research Unit (USDA-ARS SIMRU) at Stoneville, MS, to quantify CryIA(c) expression in floral structures used for insect bioassays. ELISA techniques were similar to those described by Adamczyk et al. (2000). Squares and white flowers were removed from plots of Deltapine 5415 and NuCOTN 33B. Structures were dissected into individual components as described for insect bioassays. Fifty to 100 mg of each structure were placed into 1.5-ml Eppendorf tubes and homogenized in extraction buffer. A commercial quantification plate kit (EnviroLogix, Portland, ME) was used for assays. This ELISA method utilizes color changes that are proportional to CryIA(c) concentration. Quantification of CryIA(c) was determined spectrophotometrically (Benchmark, Bio-Rad, Hercules, CA) by comparison to a standard curve. Samples were arranged in a randomized complete block design and replicated four times. Data were converted to parts per million and subjected to ANOVA (SAS Institute 1989). Means were separated according to Fisher's protected LSD. Also, correlation analyses were conducted on CryIA(c) expression and bollworm survival at each rating interval (PROC REG, SAS Institute 1989).

Bollworm Survival on Floral Components of Bollgard and Bollgard II Cottons. Plots of Bollgard II (Deltapine 50BII), Bollgard (Deltapine 50B), and conventional (Deltapine 50) cotton cultivars were planted on 11 June 2000. Bioassays conducted during 2000 with Deltapine 50, Deltapine 50B, and Deltapine 50BII used the methods described for the first experiment except they were conducted only at one growth stage (nodes above white flower 6–8). Bollworm survival within each cultivar and comparisons of bollworm survival among structures of the cultivars were subjected to repeated measures ANOVA (SAS Institute 1989), and means were separated according to Fisher's protected LSD.

Results

Bollworm Survival on Floral Components of Conventional and Bollgard Cotton. Bollworm survival varied among floral structures on Deltapine 5415 (conventional). No cotton stage by floral structure ($F < 2.08$; df = 8, 45; $P > 0.06$) or year by floral structure ($F < 0.59$; df = 4, 70; $P > 0.67$) interactions were significant at any rating interval for bollworm survival on Deltapine 5415 cotton; therefore, data were combined across cotton stages and years. Survival averaged 93–100%, 81–98%, and 71–97% at 24, 48, and 72 h after infestation, respectively (Table 1). At 24 h, bollworm survival was different among floral structures ($F = 4.37$; df = 4, 75; $P < 0.01$). Bollworm survival was

Table 2. Mean ± SD bollworm survival on Deltapine 50, Deltapine 50B (Bollgard), and Deltapine 50BII (Bollgard II) floral structures at 24, 48, and 72 h after infestation

Hours after infestation	Floral structure	Mean ± SD % survival			*F*	df	*P* > *F*
		DP 50	DP 50B	DP 50BII			
24							
	Bracts	83 ± 13Aa	80 ± 13Ba	89 ± 3Ba	0.66	2, 8	0.54
	Petals	98 ± 3Aa	100 ± 0Aa	99 ± 3Aa	0.62	2, 8	0.56
	Flower anthers	98 ± 3Aa	100 ± 0Aa	99 ± 3Aa	0.68	2, 8	0.53
	Square anthers	98 ± 3Aa	100 ± 0Aa	100 ± 0Aa	1.45	2, 8	0.29
	Squares	85 ± 6Aa	96 ± 4Aa	97 ± 4Aa	2.09	2, 8	0.19
	F	2.39	7.84	10.49			
	df	4, 10	4, 15	4, 15			
	P > *F*	0.12	<0.01	<0.01			
48							
	Bracts	67 ± 7Ca	57 ± 23Cb	29 ± 19Cb	4.16	2, 8	0.06
	Petals	95 ± 6Aa	90 ± 10ABa	81 ± 15Aa	1.28	2, 8	0.33
	Flower anthers	98 ± 3Aa	98 ± 3Aa	88 ± 17Aa	0.43	2, 8	0.30
	Square anthers	98 ± 3Aa	97 ± 3Aa	72 ± 19Ab	6.18	2, 8	0.02
	Squares	80 ± 13Ba	77 ± 12Ba	38 ± 28Bb	5.20	2, 8	0.04
	F	11.1	7.39	6.89			
	df	4, 10	4, 15	4, 15			
	P > *F*	<0.01	<0.01	<0.01			
72							
	Bracts	48 ± 9Ca	18 ± 6Db	6 ± 2Cc	42.7	2, 8	<0.01
	Petals	81 ± 9Aba	67 ± 13Ba	36 ± 21Bb	7.58	2, 8	0.01
	Flower anthers	95 ± 5Aa	93 ± 2Aa	63 ± 9Ab	33.3	2, 8	<0.01
	Square anthers	97 ± 5Aa	92 ± 3Aa	50 ± 10ABb	49.9	2, 8	<0.01
	Squares	75 ± 17Ba	49 ± 14Cb	8 ± 4Cc	25.9	2, 8	<0.01
	F	11.2	45.8	19.9			
	df	4, 10	4, 15	4, 15			
	P > *F*	<0.01	<0.01	<0.01			

Means within a column followed by the same uppercase letter and within a row followed by the same lowercase letter are not significantly ($\alpha = 0.05$) different according to Fisher's protected least significant difference.

lowest on flower bracts. Bollworm survival at 48 h ($F = 7.20$; df = 4, 75; $P < 0.01$) and 72 h ($F = 15.8$; df = 4, 75; $P < 0.01$) was higher on flower anthers and square anthers than on flower bracts and petals. Bollworm survival on anthers (flower and square) also was higher than on squares at 72 h.

Bollworm survival on NuCOTN 33B (Bollgard) cotton varied among floral structures. No cotton stage by floral structure ($F < 1.43$; df = 8, 45; $P > 0.21$) or year by floral structure ($F < 2.25$; df = 4, 70; $P > 0.07$) interactions were significant for bollworm survival at any rating interval; therefore, data were combined across cotton stages and years. Bollworm survival ranged from 85–97%, 57–96%, and 19–91% at 24, 48, and 72 h, respectively (Table 1). At 24 h, bollworm survival was lower on flower bracts than all other structures ($F = 3.94$; df = 4, 75; $P = 0.01$). At 48 h ($F = 18.9$; df = 4, 75; $P < 0.01$) and 72 h ($F = 71.3$; df = 4, 75; $P < 0.01$), bollworm survival was higher on flower anthers and square anthers than on other floral structures.

There were no differences between bollworm survival on Deltapine 5415 and NuCOTN 33B for any structure at 24 h (Table 1). At 48 h, bollworm survival was lower on NuCOTN 33B flower bracts and squares compared with the corresponding structures on Deltapine 5415. Bollworm survival was lower on all NuCOTN 33B structures compared with the corresponding structures on Deltapine 5415 at 72 h.

ELISA tests of floral structures used in these bioassays indicate that *B. thuringiensis* protein expression varies among plant parts ($F = 32.6$; df = 4, 10; $P < 0.01$). Protein expression was highest in flower bracts and petals compared with other structures. In addition, protein expression was lowest on squares and square anthers. CryIA(c) expression averaged (±SD) 0.59 ± 0.03, 0.56 ± 0.12, 0.34 ± 0.03, 0.17 ± 0.03, and 0.19 ± 0.01 ppm on flower bracts, flower petals, flower anthers, square anthers, and squares, respectively. CryIA(c) levels did not correlate (24 h: $R = -0.21$; $F = 0.63$; df = 1, 13; $P = 0.44$; 48 h: $R = -0.30$; $F = 1.33$; df = 1, 13; $P = 0.27$; 72 h: $R = -0.29$; $F = 1.18$; df = 1, 13; $P = 0.30$) with bollworm survival.

Bollworm Survival on Floral Components of Bollgard and Bollgard II Cotton. Bollworm survival on flower anthers and square anthers was generally highest and lowest on flower bracts on Deltapine 50, Deltapine 50B, and Deltapine 50BII (Table 2).

Bollworm survival on Bollgard II appeared to follow a trend similar to that observed on Bollgard. However, bollworm survival, in general, was much lower on Bollgard II than on Bollgard. At 24 h, there were no differences in bollworm survival among the three cotton cultivars on any structure (Table 2). At 48 h, bollworm survival on squares was lower on Bollgard II than on squares from the other cotton cultivars. Bollworm survival at 72 h was lower on all flower structures from Bollgard II than on the corresponding structures on the other two cotton cultivars.

Discussion

Bollworm larvae prefer specific feeding sites on cotton plants. Farrar and Bradley (1985) found that *Heliothis* larvae showed a preference for white and red flowers of conventional cotton. In that study, bollworm larvae showed a greater preference than tobacco budworm larvae for white flowers. Nonphotosynthesizing (nongreen) structures of cotton may be more common feeding sites for bollworm larvae. These structures, which are mostly reproductive, may be more nutritionally suitable for bollworm larvae than other plant parts.

Another possible explanation for bollworm preferences for flowers could be that there are lower levels of secondary plant chemicals in nonphotosynthesizing tissues. Hedin et al. (1983) reported varying levels of secondary plant chemicals (tannins, gossypol, and chrysanthemin) among different plant parts. Gossypol concentrations ranged from 0.04% in bolls to 0.50% in squares. Tannins ranged from 6.02% in terminals to 17.1% in bolls, while chrysanthemin ranged from 0.05% in bolls to 0.18% in leaves. Stipanovic (1983) reported that cotton foliage produces numerous terpenoids and other compounds in addition to gossypol. Many of the compounds found in cotton have antibiotic activity and are toxic to several insect pests. Little information is available concerning levels of secondary plant chemicals in square anthers. However, Hanny (1980) reported variation in levels of selected chemicals in flower anthers among cotton cultivars. Also, yellow flower anthers contained more gossypol than cream-colored flower anthers. Studies comparing the concentrations of secondary chemicals in flower anthers to those in other plant parts have not been conducted. It is likely that bollworm mortality on flower structures is associated with more than one allelochemical within an individual structure and differences in chemical complexes among cotton plant parts may explain the variation in bollworm survival on those plant parts.

Differences in *B. thuringiensis* CryIA(c) protein expression among different plant parts may partially explain differences in bollworm survival on those structures (Adamczyk et al. 2000). However, similar differences in bollworm survival among floral structures were observed on conventional cotton, which indicates that factors other than protein expression alone are involved. For example, interactions between plant secondary compounds and the CryIA(c) protein may have occurred. If there is an interaction between CryIA(c) and plant allelochemicals, then there would be an expected minimum critical level of protein that fluctuates based on allelochemical concentrations. For instance, structures with low allelochemical concentrations would require a higher level of CryIA(c) expression to provide the same level of bollworm mortality as structures with high allelochemical concentrations. Therefore, the interactions of these factors would be dynamic, where a decrease in one factor may require an increase in the other factor to provide the same level of protection.

Although statistical differences were observed between conventional and Bollgard cotton, bollworm survival averaged ≥88% on Bollgard flower anthers and square anthers. With this level of pest pressure, insecticide applications may be needed to prevent economic losses. Differences in bollworm survival on conventional and Bollgard cotton support the presence of CryIA(c) protein in those structures of Bollgard cotton with high levels of bollworm survival. However, expression in those structures may be low.

Bollgard II contains an additional gene that codes for the production of the CryIIA(b) protein from *B. thuringiensis* in addition to CryIA(c). The addition of the CryIIA(b) protein with CryIA(c) increased the insecticidal activity against bollworm larvae. Sims (1997) found that bollworm larvae appear to be less sensitive to CryIIA than CryIA(c). The addition of the CryIIA(b) protein into Bollgard cotton, however, would most likely increase the total amount of protein present in the plant. Greenplate et al. (2000b) measured levels of Cry proteins present in Bollgard II. They found approximately a 10 times higher level of CryIIA(b) over CryIA(c); however, there was only a three- to six-fold increase in bioactivity against tobacco budworms. In the current study, increases in bioactivity against bollworms of 3.2-, 1.6-, 1.4-, 1.8-, and 4.6-fold for flower bracts, flower petals, flower anthers, square anthers, and squares, respectively, were observed.

Bollgard cotton cultivars are valuable IPM tools for cotton systems in the United States. Good control can be expected for the tobacco budworm and pink bollworm, *Pectinophora gossypiella* (Saunders). This new technology has not always provided sufficient levels of bollworm control, however. Data reported here support field observations made by agricultural consultants and researchers throughout the southeastern United States concerning high numbers of bollworm larvae feeding on white flowers. It was originally assumed that white flowers express lower levels of CryIA(c) protein than other plant parts. However, other factors may be involved based on the ELISA data and bollworm survival trends on conventional cotton floral structures. Similar trends in bollworm survival were observed on conventional and Bollgard floral structures. Significantly fewer larvae survived on flower bracts of conventional cotton compared with survival on other conventional cotton floral structures. This finding suggests that biochemical factors associated with bracts have adverse effects on bollworm development.

The addition of a second protein into Bollgard cotton to create Bollgard II appeared to significantly increase protection against bollworms. Despite these improvements, however, bollworm survival averaged over 50% on flower anthers and square anthers of Bollgard II at 72 h. These survival rates suggest that economic injury may occur on Bollgard II during bollworm outbreaks; however, these experiments were terminated after 72 h. Our data suggest that the possibility for injury exists, but this has not been observed for Bollgard II cotton grown under field conditions.

Field studies indicate that Bollgard II cottons will consistently provide satisfactory bollworm control (Jackson et al. 2000, Ridge et al. 2000, Stewart and Knighten 2000). However, these were small plot studies conducted in relatively isolated locations and no definitive predictions can be made as to the level of bollworm protection that can expected from Bollgard II when it is planted over large acreages.

In conclusion, these data provide a baseline of information describing the levels of bollworm survival that can be expected on white flowers of Bollgard and Bollgard II cotton. This information indicates that current scouting protocols for conventional cotton may not be appropriate for Bollgard cotton. Because high levels of bollworm survival can be expected on white flowers of Bollgard cotton, those structures need to be closely examined for small larvae. Also, these data provide valuable information for improving management decisions for bollworms on Bollgard cotton. Further research is needed to determine if larvae feeding on white flowers are capable of moving to other structures, causing additional injury. Also, future research in this area should focus on quantifying secondary plant chemicals and assessing nutritional quality among selected components of white flowers and squares to determine their influence on CryIA(c) efficacy. Finally, bollworm management in genetically modified cottons (Bollgard and Bollgard II) is a complex situation that involves multiple factors. Plant biochemistry and nutrition appear to be important for bollworm mortality, in addition to *B. thuringiensis* protein expression in genetically modified cottons. Levels of secondary plant chemicals and *B. thuringiensis* protein expression need to be determined for different genetically modified cultivars and among different plant parts so that bollworm survival can be predicted during periods of high population densities.

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